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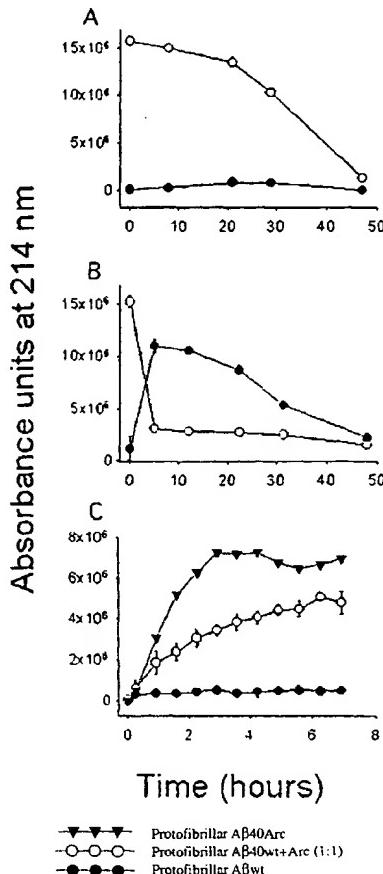
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(54) Title: PREVENTION AND TREATMENT OF ALZHEIMER'S DISEASE



(57) Abstract: The present invention relates to prevention and treatment of Alzheimer's disease (AD). More specifically, the invention relates to use of a non-wild type protofibril or compound(s) with protofibril forming activity for active immunisation in the purpose of treating or preventing AD. The invention further relates to a peptide,  $\alpha\beta$ -Arc, with high protofibril forming activity as well as several applications thereof, such as antibodies against said peptide for passive immunisation against AD.

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Title: Prevention and treatment of Alzheimer's disease

### Field of the invention

The present invention relates to prevention and treatment of Alzheimer's disease (AD). More specifically, the invention relates to use of a non-wild type protofibril or compound(s) with protofibril forming ability for active immunisation in the purpose of treating or preventing AD. The invention further relates to a peptide, A $\beta$ -Arc, with high protofibril forming activity as well as several applications thereof, such as antibodies against said peptide for passive immunisation against AD.

### Background of the invention

Alzheimer's disease (AD) is a progressive disease known generally as senile dementia. The disease falls into two categories, namely late onset and early onset. One form of this latter AD type runs in families and it is known as familial AD.

Both types of AD are characterized by two types of lesions in the brain: senile plaques and neurofibrillary tangles. Senile plaques are areas of disorganized neuropil up to 150 mm across with extracellular amyloid deposits at the center. Neurofibrillary tangles are intracellular deposits consisting of two filaments twisted about each other in pairs.

A $\beta$  also referred to as amyloid  $\beta$  peptide (A $\beta$ P) is a highly aggregating small polypeptide having a molecular weight of approximately 4,500. This protein is a cleavage product of a much larger precursor protein referred to as amyloid precursor protein (APP). The A $\beta$  protein comprises 39 - 42 amino acids. There are at least five distinct isoforms of APP: 563, 695, 714, 751, and 770 amino acids, respectively (Wirak et al. (1991)). The A $\beta$  protein segment comprises approximately half of the transmembrane domain and approximately the first 28 amino acids of the extracellular domain of an APP isoform.

APP is a transmembrane protein which is highly expressed in all parts of the body, and which has several important biological functions. Proteolytic processing of APP in vivo is a normal physiological process. Carboxy-terminal truncated forms of APP695, APP751, and APP770 are present in brain and cerebrospinal fluid (Palmer et al. (1989)) (Weidemann et al (1989)). There are probably two main metabolic pathways: one non-amyloid-forming and one amyloid-forming pathway. The amyloid forming non-normal pathway produces the A $\beta$  protein polypeptide which is prone to form dense amyloidogenic aggregates that

are resistant to proteolytic degradation and removal. The resultant A $\beta$  protein aggregates presumably are involved in the formation of the abundant amyloid plaques and cerebrovascular amyloid that are the neuropathological hallmarks of AD.

In AD brains, the A $\beta$  peptide forms virtually insoluble amyloid fibrils that accumulate into senile plaques. The A $\beta$  fibrillization process is a complex multistep reaction. A group of distinct intermediary A $\beta$  species of the fibrillization reaction, the protofibrils, were recently identified (Walsh et al. (1997)), (Walsh et al. (1999)), (Harper et al.(1999)).

The most common A $\beta$  form in cerebrospinal fluid (CSF) and plasma comprises 40 amino acids (A $\beta$ 40), but an A $\beta$  comprising 42 amino acids (A $\beta$ 42) is the most common form in plaques (Scheuner et al. (1996)). This longer form tends to aggregate more rapidly and it is believed that it is more pathogenic than A $\beta$ 40.

Many patients get Alzheimer's disease spontaneously with unknown ethiology, but there are also several hereditary components involved. Disease-causing mutations in genes on chromosomes 1, 14, and 21, respectively, have been discovered, and these mutations might explain as much as 50% of disease forms starting very early (<50 years)(St. George-Hyslop et al. (1987), (Sherrington et al. (1995)).

The first gene associated with Alzheimer's disease was the gene encoding the amyloid precursor protein APP on chromosome 21. Different mutations of this gene result in unusual hereditary forms of the disease. Several pathogenic mutations have been identified in the (APP) gene, all located close to the major APP processing sites. These processing sites are either located adjacent to the boundaries of the A $\beta$  domain in APP (the  $\beta$ - and  $\gamma$ -secretase sites) or within the A $\beta$  sequence itself ( $\alpha$ -secretase site).

The only known AD mutation close to the  $\beta$ -secretase site, the Swedish mutation (Mullan, et al.,(1992)), discloses a double mutation (Lys670Asn/Met671Leu) of the APP gene in a large Swedish family, in which family the disease starts early and has a high penetrating power. The mutation produces a large increase of A $\beta$  production, an elevation of both A $\beta$ 42 and A $\beta$ 40 in plasma from mutation carriers and in conditioned cell media.

Other APP mutations have been described. All result in Alzheimer's disease with an early age of onset having an autosomal dominant heredity pattern. Pathogenic mutations within the A $\beta$  sequence, located close to the  $\alpha$ -secretase site, result in a phenotype different from AD, with massive amyloid accumulation in cerebral blood vessel walls. Two mutations at codons 692 and 693, namely the Dutch (Glu693Gln) and the Flemish (Ala692Gly) mutations, have been reported (Levy et al. (1990)), (van Broeckhoven et al. (1990)), (Hendriks et al. (1992)). Patients having these mutations suffer from cerebral haemorrhage and vascular symptoms. The vascular symptoms are caused by aggregation of A $\beta$  in blood vessel walls (amyloid angiopathy). A third pathogenic intra-A $\beta$  mutation was recently discovered in an Italian family (E693K), with clinical findings similar to the Dutch patients (Tagliavini, et al. (1999)).

Different pathogenic mechanisms have been proposed for the Dutch and Flemish mutations. It has been observed that the Flemish mutation leads to increased A $\beta$  levels while a reduced ratio of A $\beta$ 42/40 was seen in media from cells transfected with the Dutch mutation (De Jonghe, et al.(1998)). Investigations of synthetic A $\beta$  peptides have indicated that the Dutch mutation, but not the Flemish, accelerates the fibril formation compared to wild-type (wt) peptide (Walsh et al. (1997)).

As reported by Kamino et al. 1992, another APP E693 variant wherein Glu is substituted for Gly at APP E693, has previously been seen in one individual. It could not be unambiguously determined to be responsible for AD, though. This case originated from a family with similar clinical characteristics for AD and definitive AD was confirmed at autopsy. However, in this family the mutation could only be detected in one of two demented siblings.

Mice transgenic for APP mutations show many of the pathological features of Alzheimer disease, including deposition of extracellular amyloid plaques, astrocytosis and neuritic dystrophy. In recent studies by (Schenk et al (1999)) it was reported that immunization with A $\beta$ 42 wild-type peptide is both preventive in transgenic mice, but also that A $\beta$  containing plaques can be greatly reduced in the brain of transgenic mice immunized with the peptide.

However, due to the large costs and suffering that are associated with Alzheimer's disease, there is still a need for improved methods for treatment and prevention thereof.

Likewise, there is a need for a method for screening compounds that could constitute a part of future pharmaceutical preparations for treating and perhaps curing Alzheimer's disease.

### Summary of the invention

The present invention relates to an active immunisation against AD which will have a much more profound effect in the treatment of Alzheimer's disease, than using the wild-type peptide. Immunization according to the invention will yield antibodies directed to protofibrils, as the immunogen is a protofibril or compound(s) with greatly increased protofibril formation properties. These antibodies, generated in the periphery, will cross the blood brain barrier and mediate clearance of A $\beta$  in the brain in a protofibril state.

In present invention use is made of a pathogenic AD mutation at codon 693 (Glu693Gly), named the 'Arctic mutation', located within the A $\beta$  peptide domain of the APP gene, more closely position 22 of the A $\beta$ -Arc peptide. Carriers of this mutation develop progressive dementia with clinical features typical of AD without symptoms of cerebrovascular disease. Said AD is distinctly characterised by accelerated formation of protofibrils comprising mutated A $\beta$  peptides (40Arc and/or 42Arc) compared to protofibril formation of wild type A $\beta$  peptides.

Thus, in a first aspect the invention relates to use of a non-wild type protofibril or compound(s) with protofibril forming ability for immunisation for prevention or treatment of Alzheimer's disease (AD). Preferably, these protofibril or compound(s) have enhanced protofibril forming ability and/or enhanced immunogenicity compared to the wild-type counterparts. Protofibril chemistry has been described by, inter alia, Serpell (2000).

Preferably, the protofibril or compound(s) with protofibril forming ability comprises the following amino acid sequence KLVFFAEDV. The A $\beta$  1-42 fibrillisation process involves transitional conformation changes from  $\alpha$ -helix via random coil to  $\beta$ -sheet. The stable  $\alpha$ -helix sequence of residues 16-24 (KLVFFAEDV) apparently plays an important role in this process.

The protofibril or compound(s) with protofibril forming ability may be mutated or modified in relation to corresponding wild-type counterparts. Changes in the KLVFFAEDV

sequence will affect the fibrillisation process. For example, changes of the charged amino acids Glu22 and Asp23 into neutral amino acids will induce a random coil structure in the A $\beta$  peptide. Furthermore, deprotonation of other amino acids such as Asp7, Glu11 and His 6, 13 and 14 in the N-terminal end, has been suggested to destabilize the  $\alpha$ -helix, leading to initiation of the fibrillation process. Another example is mutations leading to increased immunogenicity in man by using amino acids from mouse A $\beta$  at specific positions, e.g. Gly 5, Phe10, Arg13. Furthermore, amino acid 13 in A $\beta$  is known to be part of a heparan sulphate binding motif (13-16; His, His, Gln, Lys) in human, which has been speculated to be involved in AD disease mechanism (inflammation) (Giulian et al. (1998)). In mouse, His 16 is exchanged for Arg 13 destroying the heparan sulphate binding site. Interestingly, mice have never been observed to develop AD. Hence, the use of A $\beta$ -Arc/Arg13 as an immunogen would be a way to lower possible inflammatory side effects, elicited with A $\beta$  peptides with intact heparan sulphate binding motif.

Preferably, the protofibril or compound(s) with protofibril forming ability comprises an A $\beta$  peptide ( $\beta$ -amyloid protein) and repeats thereof, such as dimeric, oligomeric or multimeric forms). In a preferred embodiment the protofibril or compound(s) with protofibril forming ability comprises a A $\beta$  peptide related to AD. In another embodiment the protofibril or compound(s) with protofibril forming ability comprises  $\alpha$ -synuclein.

There exists a form of dementia characterised by patients having clusters in the brain of a structure called Lewy bodies. This form of dementia comprises about 20% of all dementia. Patients with Lewy bodies show, inter alia, Parkinson symptoms with progressive cognitive dysfunction. However, some patients also exhibit Alzheimer symptoms and this is called "Lewy variant of Alzheimer". The main component of the Lewy bodies is the protein  $\alpha$ -synuclein. Two mutations in  $\alpha$ -synuclein have been identified Ala53Thr and Ala30Pro. These mutations lead to dominant heritage of Parkinson's disease. These mutations affect the structure/solubility of  $\alpha$ -synuclein and leads to formation of protofibrils. (Conway et al. (2000)).

The A $\beta$  peptide is preferably A $\beta$ -Arc as disclosed in SEQ ID NO 1. A $\beta$  -Arc comprises 39, 40 or 42 amino acids but may also be shorter as long as the protofibril forming ability is maintained.

The profibril or compound(s) with protofibril forming ability may be used in combination with A $\beta$  peptides having known mutations, such as the Dutch, Flemish, Italian mutation described above as well as the Iowa mutation (D694N) (Grabowski et al., 2001).

The A $\beta$  peptide may comprise one or more of these and/or other mutations. Alternatively, a cocktail of different A $\beta$  peptides with different mutations is used.

In a second aspect, the invention relates to a peptide, A $\beta$ -Arc, having the amino acid sequence disclosed in SEQ ID NO 1 comprising a glycine at position 22 instead of glutamic acid compared to wild type A $\beta$  peptide. The peptide may be natural, synthetic or recombinantly produced. For the purposes of the invention the peptide may be used in monomeric, dimeric, oligomeric, protofibril or multimeric form.

The invention also relates to nucleic acid encoding the above peptide as well as a vector comprising the nucleic acid. The vectors for expressing the polypeptides of the invention require that the nucleic acid be "operatively linked." A nucleic acid is operatively linked when it is placed into a functional relationship with another nucleic acid sequence.

This vector may be inserted in a host cell. Such a host cell can be used to recombinantly produce the peptide of the invention for pharmaceutical or diagnostic use as well for research purposes. The peptide may also be produced synthetically and be purified by HPLC, RP-HPLC, SEC-HPLC.

In a further aspect, the invention relates to a transgenic non-human animal comprising the above vector. Furthermore, the invention relates to a transgenic non-human animal comprising a vector comprising the entire APP gene corresponding to NCBI database, accession no XM\_009710, Homo sapiens amyloid  $\beta$  (A4) precursor protein (protease nexin-II, Alzheimer's disease)(APP), mRNA. However, the APP gene for use in the invention comprises the Arctic mutation, i.e. nucleotide number 2225 is mutated from A to G leading to an amino acid substitution from Glutamic acid to Glycine. The transgenic animal may be used for modelling Alzheimer's disease and testing for therapeutic treatment efficacy. This transgenic animal will bear the entire APP gene comprising the Arctic mutation. This gene is preferably under control of a strong promoter, such as the prion-promoter. The APP gene may contain further mutations, besides the Arctic mutation.

The transgenic animal expresses a human APP or a fragment thereof which encodes glycine instead of glutamic acid at codon 693. Preferably, the animal expresses neuropathological characteristics of AD. Preferably, the mutated APP is expressed in cells which normally expresses the naturally-occurring endogenous APP gene (if present). Typically, the non-human animal is a mouse. Such transgenes typically comprises an Arctic mutation APP expression cassette, wherein a linked promoter and, preferably, an enhancer drive expression of structural sequences encoding a heterologous APP polypeptide comprising the Arctic mutation.

Such transgenic animals are usually produced by introducing the transgene or targeting construct into a fertilized egg or embryonic stem (ES) cell, typically by microinjection, electroporation, lipofection, or biolistics. The transgenic animals express the Arctic mutation APP gene of the transgene (or homologously recombined targeting construct), typically in brain tissue. Alzheimer phenotype and neuropathology is caused by protofibril formation. Such animals are suitable for use in a variety of disease models and drug screening uses, as well as other applications.

In yet a further aspect, the invention relates to antibodies against the A<sub>β</sub> peptide of SEQ ID NO 1. The antibodies may be monoclonal or polyclonal or antibody fragments. Preferably the antibodies are humanized for use in passive immunisation for prevention or therapy against AD. Thus, antibodies which react with the unique epitope created by glycine at codon 693 are provided.

Another aspect of the invention relates to a pharmaceutical composition, comprising the above peptide and physiologically acceptable excipients for human and veterinary use. The preparation may comprise adjuvants for vaccination purposes. The administration route may be s.c., i.m., oral or nasal.

In a further aspect, the invention relates to use of the above A<sub>β</sub> peptide for high throughput screening to find substances with anti-protofibrillar activity.

In a further aspect, the invention relates to a method for prevention or treatment of AD, comprising the step:  
decreasing the formation of A<sub>β</sub> protofibrils and/or lower meric forms thereof in a subject having, or suspected of having, AD.

The decreasing step above may be by active immunisation with a profibril or compound(s) with protofibril forming ability for prevention or treatment of Alzheimer's disease (AD), wherein said protofibril or compound(s) have enhanced protofibril forming ability and/or enhanced immunogenicity compared to the wild-type counterparts.

Alternatively, the decreasing step above is by passive immunisation with antibodies against protofibrils or compound(s) with protofibril forming ability, such as A $\beta$ -Arc. The passive immunisation may be in combination with antibodies against other A $\beta$  peptides with mutations/modifications leading to increased protofibril formation and/ or immunogenicity, preferably AD related mutations.

Antibodies generated against the human A $\beta$  sequence containing the Arctic mutation are directed towards A $\beta$  protofibrils and therefore are of therapeutic value in the treatment of Alzheimer's disease. Because the A $\beta$  peptide is in a protofibril conformation when used as an immunogen, antibodies against A $\beta$  protofibrils are generated. Availability of such antibodies opens up possibilities for the development of an efficient and lasting vaccination for the prevention and treatment of Alzheimer's disease.

In another alternative the decreasing step of the method according to the invention is by administration of agents with anti-protofibrillar activity.

In yet a further aspect of the invention, a combination of the vaccine or passive immunization with monoclonal antibodies or compounds with anti-fibrillar activity with one or several other AD treatments such as, acetylcholinesterase inhibitors, nootropics, anti-inflammatory drugs, estrogen, neurotrophic factor agonists,  $\beta$ -secretase inhibitors,  $\gamma$ -secretase inhibitors and  $\alpha$ -secretase agonists, can improve AD treatment efficacy. The rational is that these substances/treatments work with completely different mechanisms of action and hence can be combined to the benefit for the AD patient.

#### **Detailed description of the invention**

The basis of the present invention is a pathogenic amyloid precursor protein (APP) mutation located within the A $\beta$  sequence at codon 693 (E693G), causing AD in a family from northern Sweden. Surprisingly, carriers of this "Arctic" mutation show decreased A $\beta$ 42 and A $\beta$ 40 levels in plasma. This finding is corroborated *in vitro*, where the A $\beta$ 42

concentration was low in conditioned media from cells transfected with APP<sub>E693G</sub>. Fibrillization studies demonstrate that A $\beta$  peptides with the Arctic mutation (A $\beta$ 40Arc) form protofibrils at a much higher rate and in larger quantities than wild-type (wt) A $\beta$  (A $\beta$ 40wt). The unique finding of decreased A $\beta$  plasma levels in the Arctic AD family highlights the complexity of the disease and is likely to reflect a novel pathogenic mechanism. The mechanism disclosed in the present invention involves a rapid A $\beta$  protofibril formation leading to accelerated build-up of insoluble A $\beta$  intra- and/or extracellularly.

In the present invention, the single amino acid substitution Glu to Gly at position 22 in the A $\beta$ 4040Arc molecule was found to cause a dramatic increase in rate and capacity to form protofibrils compared to the A $\beta$ 40wt peptide. Thus, when A $\beta$ 42Arc and A $\beta$ 40Arc are formed in the brain it is likely that they are more prone to be retained by cellular systems since the accelerated drive to form protofibrils enhances both A $\beta$  bulk and insolubility. Thus, factors promoting protofibril formation should be considered in the pathogenesis of sporadic AD. Increased protofibril formation is probably also operating in these more common forms of the disease. Indeed, the findings of the present invention open new avenues for possible therapeutic intervention using drugs targeted at preventing protofibril formation.

Studies on the Arctic mutation of the present invention have demonstrated a previously not described pathogenic mechanism for Alzheimer's disease through increased formation of A $\beta$  protofibrils. A $\beta$  with the Arctic mutation formed more stable protofibrils and at a much higher rate and in larger quantities than wild-type A $\beta$ , even in the presence of equimolar amounts of wild-type A $\beta$ . The formation is accelerated at least 2-10 times compared to protofibrill formation of wild type A $\beta$  peptides. The implication of this finding is that the dangerous species in the amyloid forming pathway that eventually leads to Alzheimer's disease is not the A $\beta$  fibrils, but a form of the peptide that appears earlier in the fibril maturation process, the protofibrils. One implication of the findings related to the present invention is that it is important to prevent the formation of protofibrils in order to be able to prevent and treat Alzheimer's disease.

Non-human animals comprising transgenes which encode Arctic mutation APP can be used commercially to screen for agents having the effect of lowering the formation of A $\beta$  protofibrils. Such agents can be developed as pharmaceuticals for treating abnormal APP processing and/or Alzheimer's disease, amongst other neurodegenerative conditions in

humans and animals, such as dogs. The transgenic animals of the present invention exhibit abnormal APP processing and expression, and can be used for pharmaceutical screening and as disease models for neurodegenerative diseases and APP biochemistry.

### Figure legends

The present invention will now be further described with reference to the enclosed figures, in which:

Figure 1 shows kinetics of soluble forms of A $\beta$ 1-40wt (a), A $\beta$ 1-40Arc (b) and protofibril formation of A $\beta$ 1-40wt, A $\beta$ 1-40Arc vs a mixture of A $\beta$ 1-40wt + Arc (1:1) (c). The A $\beta$ 1-40Arc peptide (92  $\mu$ M) rapidly forms protofibrils (black dots) in comparison to the A $\beta$ 1-40wt peptide (88  $\mu$ M), which mainly is in monomeric(dimeric (grey dots) form, data is taken from one experiment, representative of three (a and b). The protofibril formation rate was monitored during the first seven hours and the kinetics for the pure peptides (A $\beta$ 1-40wt and A $\beta$ 1-40Arc at 50  $\mu$ M) was compared to the protofibril formation rate of a 1:1 mixture (50  $\mu$ M) of A $\beta$ 1-40wt + Arc (c).

Figure 2 depicts elution profiles showing A $\beta$ 40wt (a-c) versus A $\beta$ 40Arc (d-f) at 5 (a,d), 45 (b,e) and 125 (c,f) min of incubation. Accelerated protofibril (p) formation along with a parallel decline in the monomeric/dimeric (m/d) A $\beta$  levels could be observed for A $\beta$ 40Arc (d-f) as compared to A $\beta$ 40wt (a-c). Data is from one experiment, representative of four. Initial peptide concentrations were 143  $\mu$ M and 138  $\mu$ M for A $\beta$ 40wt and A $\beta$ 40Arc, respectively.

### EXAMPLES

The following examples are provided for illustration and are not intended to limit the invention to the specific example provided.

#### ***Example 1: Identification of the Arctic mutation***

An APP mutation (E693G) in a family from northern Sweden, named the "Arctic" family, is identified, which spans over four generations. The family was screened for mutations in exons 16 and 17 of the APP gene by single strand conformation polymorphism analysis (SSCP) (L. Forsell, L. Lannfelt, (1995)). An abnormal mobility pattern was observed in

exon 17. Sequencing revealed an A→G nucleotide substitution, representing a glutamic acid to a glycine substitution at APP codon 693 (E693G), corresponding to position 22 in the A $\beta$  sequence. Venous blood was drawn into tubes containing EDTA and DNA was prepared according to standard procedures. SSCP was performed. To sequence exon 17 of the APP gene a 319 bp fragment was amplified with the following primers 5'-CCT CAT CCA AAT GTC CCC GTC ATT-3' and 5'-GCC TAA TTC TCT CAT AGT CTT AAT TCC CAC-3'. The PCR products were purified with QIAquick PCR purification kit (Qiagen) prior to sequencing. Direct sequencing was performed in both 3' and 5' direction using the same primers and the BIG Dye cycle sequencing protocol (PE Biosystems) and were then analyzed on an ABI377 automated sequencer (PE Biosystems). The Arctic mutation was seen in one family and not in 56 controls or 254 cases with dementia. Carriers of the arctic mutation showed no vascular symptoms. The mutation was further verified by restriction analysis, since it destroyed a MboII restriction site. The mutation was fully penetrant as no escapees were found. Two-point linkage analysis was performed between the mutation and affection status in the family with an age-dependent penetrance, giving a lod score of 3.66 at recombination fraction 0.00. Two-point lod score was calculated using Mlink from the linkage package (version 5.1) at each of the following recombination fractions 0.00, 0.10, 0.20, 0.30 and 0.40 (q males=q females). A single-locus model with an autosomal dominant inheritance was assumed, which was compatible with the inheritance as it appeared in the pedigree. A cumulative age dependent penetrance was assigned from the known ages of onset in the family. Individuals were put into different liability classes depending on the age at onset (affected) or age at last examination (unaffected). The disease gene frequency and the marker allele frequency were estimated to be 0.001 and the phenocopy rate was set to 0.0001.

#### ***Example 2: Clinical symptoms in carriers of the Arctic mutation***

The family with the "Arctic" mutation was clinically and genealogically investigated. In this family, the mean age of onset was 56.6 years and the mean duration of the disease was 7 years (n=5).

The first symptom in most cases in this family was an insidious loss of memory for recently acquired information. Symptoms before clinical manifestation of Alzheimer's disease were decreased power of concentration and difficulties in handling stress situations. All affected individuals in generation IV had an early retirement pension because of the disease. The patients in generation IV were investigated by magnetic

resonance imaging (MRI), computed tomography (CT) and electroencephalography (EEG) which confirmed the diagnosis of Alzheimer's disease. In four individuals CT and MRI did not demonstrate signs of stroke or cerebral haemorrhage.

***Example 3: Decreased A $\beta$  plasma levels in carriers of the Arctic mutation***

Pathogenic APP mutations have been shown to affect APP processing, as reflected in an increase of either total A $\beta$  or A $\beta$ 42 in the plasma of affected family members. The Arctic mutation is located in a region different from other AD-causing mutations. It was investigated as to whether the mutation manifested itself by affecting A $\beta$  plasma levels. Plasma from nine mutation carriers, of which four were symptomatic, and eleven non-carriers in the family, were analysed by well-characterized sandwich ELISA systems, specifically detecting A $\beta$ 42 (BAN50/BC05) and A $\beta$ 40 (BAN50/BA27) (Suzuki et al. 1994)). To reassure that the Arctic mutation did not change any of the antibody recognition sites A $\beta$ 40wt and A $\beta$ 40Arc peptides were tested and found to be recognized equally well. Furthermore, plasma was spiked with synthetic peptides, revealing that both A $\beta$ Arc and A $\beta$ wt peptides were recovered by ELISA to the same extent. The data obtained was analyzed by non-parametric Mann-Whitney analysis. The A $\beta$ 42 plasma concentration was  $11.7 \pm 3.9$  fmol/ml and  $16.0 \pm 5.6$  fmol/ml in mutation carriers and non-carriers, respectively, representing a 27% reduction of A $\beta$ 42 in the mutation carriers ( $p=0.04$ ). The A $\beta$ 40 plasma concentration was  $105 \pm 22$  fmol/ml and  $141 \pm 34$  fmol/ml in mutation carriers and non-carriers, respectively, representing a 26% reduction of A $\beta$ 40 in the mutation carriers ( $p=0.01$ ). The A $\beta$ 42/40 ratio was calculated for each individual, but no significant difference was found ( $p=0.13$ ). In conclusion, concentrations of both A $\beta$ 42 and A $\beta$ 40 were unexpectedly and significantly reduced in individuals carrying the Arctic mutation.

***Example 4: A $\beta$  levels in cell culture***

The effect of the Arctic mutation on A $\beta$  formation was further investigated *in vitro* in transiently transfected HEK293 cells. APPwt was compared to the following mutations: Arctic (APP<sub>E693G</sub>), Dutch (APP<sub>E693Q</sub>), Italian (APP<sub>E693K</sub>) and Flemish (APP<sub>A692G</sub>). Constructs containing the Swedish double mutation (APP<sub>Swe</sub>) and one APP mutation at codon 717 (APP<sub>V717F</sub>), both with well-studied APP processing characteristics (Hardy (1997)), were used as positive controls. The mutations were introduced to APP695 cDNA in pcDNA3 using QuikChange™ Site-Directed Mutagenesis Kit according to the manufacturers

instructions (Stratagene). The mutated constructs were verified by sequencing. For the ELISA measurements, HEK293 cells were seeded in six-well dishes and transfected with the different constructs using FuGENE™ 6 Transfection Reagent (Roche Diagnostics) according to the manufacturers instructions. 24 h after transfection, the cells were conditioned 48 h in OptiMEM containing 5% newborn calf serum. After withdrawal of the media for ELISA measurements, the APP expression in the cells were investigated by western blot using monoclonal antibody 22C11 (Roche Diagnostics). Media was conditioned and analyzed for A $\beta$  levels by the same A $\beta$ 42- and A $\beta$ 40-specific sandwich ELISA systems as used for human plasma (Citron, et al. (1997)). The A $\beta$ 42 and A $\beta$ 40 concentrations and A $\beta$ 42/40 ratios are shown in Table 1.

Table 1 A $\beta$ 42/40 ratio and A $\beta$ 42 and A $\beta$ 40 levels in conditioned media from transiently transfected HEK293 cells

APP constructs	A $\beta$ 42/40 ratio (%) $\pm$ SD	A $\beta$ 42 $\pm$ SD (fmol/ml)	A $\beta$ 40 $\pm$ SD (fmol/ml)
APPwt	9.6 $\pm$ 0.7	13.8 $\pm$ 1.0	144 $\pm$ 6
Arctic (E693G)	7.5 $\pm$ 0.5*	11.2 $\pm$ 0.6	149 $\pm$ 3
Dutch (E693Q)	6.6 $\pm$ 0.6*	9.6 $\pm$ 0.7	147 $\pm$ 12
Italian (E693K)	6.4 $\pm$ 0.6*	8.0 $\pm$ 0.7	126 $\pm$ 17
Flemish (A692G)	11.7 $\pm$ 1.6*	27.0 $\pm$ 2.0	232 $\pm$ 25
Mock (vector only)	7.2 $\pm$ 2.4	2.1 $\pm$ 1.0	28 $\pm$ 5

\* P=0.004 in comparison to APPwt

Decreasing A $\beta$ 42/A $\beta$ 40 ratios could be seen with all mutations at APP 693 (Arctic, Dutch, Italian). This may be due to increased rate of intracellular protofibril formation.

#### ***Example 5: Effect of Arctic mutation on protofibril formation***

The effect of the single amino acid substitution (Glu22Gly) on amyloid fibrillization kinetics was investigated. Synthetic A $\beta$ 1-40 was dissolved in physiological buffer and incubated for different periods of time. After centrifugation, the soluble A $\beta$  in the supernatant, both low molecular weight (monomeric/dimeric) A $\beta$  and protofibrils, were separated and analyzed using size exclusion chromatography (SEC) with UV detection at 214 nm. The morphology of the sedimented insoluble A $\beta$  was visualized using negative stain and transmission electron microscopy (TEM).

A $\beta$ 1-40wt was purchased from Bachem, Bubendorf, Switzerland or Biosource International/QCB (Camarillo, CA, USA) and A $\beta$ 1-40Arc from Biosource International/QCB. The peptides were trifluoroacetic salts. They were stored at -20°C. All other chemicals were of highest purity available. Samples of each peptide were incubated, without agitation, at 30°C in 50 mM Na<sub>2</sub>HPO<sub>4</sub> · NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4) containing 0.1 M NaCl, for various time-points. Initial peptide concentrations were within the range of 88-143  $\mu$ M, and were similar for both peptides in each experiment. After centrifugation (17 900 x g for 5 min at 16°C) monomeric/dimeric and protofibrillar A $\beta$ 1-40, sampled from the supernatant, were separated using SEC. A Merck Hitachi D-7000 LaChrom HPLC system, having a diod array detector model L-7455, a L-7200 model autosampler and a model L-7100 pump, coupled to a Superdex 75 PC3.2/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden), was used for the chromatographic separation and analysis. Samples were eluted at a flow rate of 0.08 ml/min (ambient temperature) using 50 mM Na<sub>2</sub>HPO<sub>4</sub> NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 0.15 M NaCl. Chromatograms were obtained by measuring UV absorbance at 214 nm. Peak areas for monomeric/dimeric and protofibrillar A $\beta$  were integrated using Merck-Hitachi Model D-7000 Chromatography Data Station Software. The mean of triplicate integrated peak values from the SEC measurements were used to generate each data point shown in Fig. 1 and 2. In addition, a standard curve was produced by correlating integrated peak areas with peptide concentrations as determined by quantitative amino acid analysis. The concentrations of total (at t=0 h) and soluble peptides remaining in solution after centrifugation were calculated from the standard curve.

SEC analysis of freshly dissolved A $\beta$ 1-40wt generated a single elution peak at a retention time of about 20 min (Fig. 2a). This peak represented the monomeric/dimeric forms of A $\beta$ 1-40wt (Walsh et al.(1997)). With increasing incubation time a second distinct peak appeared in the gel-excluded fraction with a retention time of about 12 min. This earlier peak contained protofibrils (Fig. 2b, c), as verified by ultracentrifugation, negative stain and TEM of A $\beta$ 1-40wt (data not shown), in line with previous findings (Walsh et al. (1997)). Similar retention times were obtained for the A $\beta$ 1-40Arc peptide (Fig. 2d-f). However, A $\beta$ 40Arc generated protofibrils much faster and in larger quantities than A $\beta$ 40wt. Chromatograms from three early time-points of incubation illustrate this difference (Fig.1). The monomeric/dimeric A $\beta$ 40Arc peak declined in parallel with the growth of the

protofibrillar peak (Fig. 2d-f). The maximum concentration (111 µM) of Aβ40Arc protofibrils was observed at 6.5 h.

Kinetic studies up to 48 h showed that Aβ1-40wt generated a small quantity of protofibrils with a maximum concentration at 25 h (Fig. 1a). In contrast, a rapid and significant formation of protofibrils was seen within the first 5 h of incubation with a simultaneous rapid decline in the concentration of the monomeric/dimeric Aβ1-40Arc peptide (Fig. 1b). Since carriers of the Arctic mutation are heterozygots they generate both Aβwt and AβArc. Assuming equimolar in vivo production, the kinetics of protofibril formation was studied in a 1:1 mixture of Aβ1-40wt and Aβ1-40Arc. This mixture of peptides showed kinetics that were intermediate to the single peptide curves (Fig. 1c).

#### ***Example 6: Morphology of Aβ-Arc***

A typical fibrillar morphology of Aβ1-40Arc in sedimented samples from kinetic studies was confirmed by negative stain and TEM. Aβ peptide samples were prepared and incubated as indicated for the kinetic studies, using higher peptide concentrations (617 µM). After 8 days, aggregated Aβ species were sedimented using the same centrifugation parameters as described above. Buffer was removed and pelleted material was suspended in 50 µl water using gentle sonication (2 x 6s). Eight µl samples were applied to carbon stabilized Formvar film grids (Ted Pella, Inc., Redding, CA, USA). Samples were negatively stained with 8 µl uranyl acetate (1%) (E. Merck, Darmstadt, Germany). Four grids were prepared for each sample and examined using a Philips CM10 TEM. Samples from pellets sedimented during the kinetic experiments were also examined. Similar to the sedimented Aβ40wt, large mesh-works of Aβ could be seen in these preparations. Protofibrils could also be discerned in the sedimented samples. The Aβ1-40Arc protofibrils were longer and less curved compared to the Aβ1-40wt protofibrils. Inter-twining of several fibrils was more common in the Aβ40Arc preparations, resulting in larger fibril diameters.

#### ***Example 7: Kinetic studies***

Kinetic studies comparing the formation of Aβ40gly22 protofibrils in the presence of a high and a low concentration of NaCl:

The experiments examining Aβ40gly22 protofibril and fibril formation, have been performed in 50 mM phosphate buffer supplemented with 100 mM NaCl. They present

data that show that the rate and magnitude of A $\beta$ 40gly22 protofibril formation is significantly enhanced in the presence of a high NaCl concentration.

Since intra- and extraneuronal NaCl concentrations differ significantly (ca117 mM vs 30mM), this finding supports an increased ability of A $\beta$ 40gly22 to form protofibrils in the extra-neuronal space where  $\beta$ -amyloid plaques are found.

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<110> Lannfelt, Lars

<120> Prevention and treatment of Alzheimer's disease

<130> P05384PC

<210> 1

<211> 42

<212> PRT

<213> Homo sapiens

<400> 1

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1

5

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15

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25

30

Met Val Gly Gly Val Val Ile Ala

35

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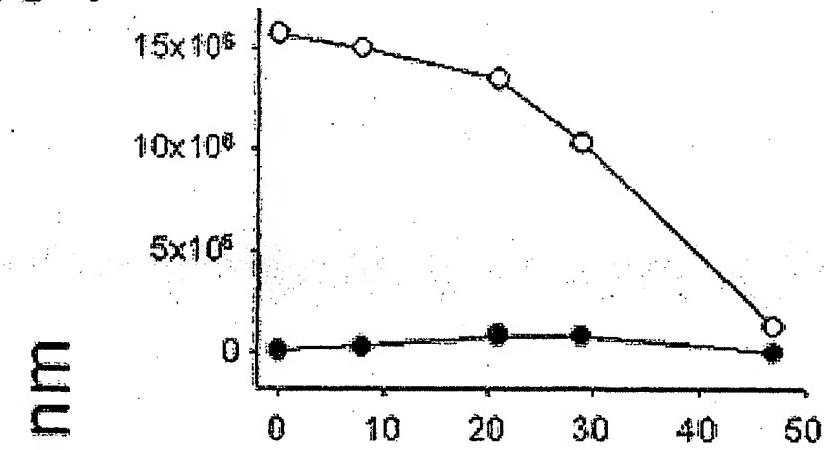
## CLAIMS

1. Use of a non-wild type protofibril or compound(s) with protofibril forming ability for immunisation for prevention or treatment of Alzheimer's disease (AD).
2. Use according to claim 1, wherein said protofibril or compound(s) with protofibril forming ability comprises the following amino acid sequence KLVFFAEDV.
3. Use according to claim 1 or 2, wherein said protofibril or compound(s) with protofibril forming ability is mutated or modified in relation to corresponding wild-type counterparts.
4. Use according to claim 1, 2 or 3, wherein said protofibril or compound(s) with protofibril forming ability comprises an A $\beta$  peptide ( $\beta$ -amyloid protein).
5. Use according to claim 4, wherein said protofibril or compound(s) with protofibril forming ability comprises a A $\beta$  peptide related to AD.
6. Use according to claim 5, which is A $\beta$ -Arc as disclosed in SEQ ID NO 1.
7. Use according to any of the above claims, wherein said profibril or compound(s) with protofibril forming ability is used in combination with A $\beta$  peptides having mutations.
8. A peptide A $\beta$ -Arc having the amino acid sequence disclosed in SEQ ID NO 1 comprising a glycine at position 22 instead of glutamic acid compared to wild type A $\beta$  peptide.
9. Nucleic acid encoding the peptide according to claim 8.
10. Vector comprising the nucleic acid according to claim 9.
11. Host cell comprising the vector according to claim 10.
12. Transgenic non-human animal comprising the vector according to claim 10.

13. Transgenic non-human animal comprising a vector comprising the entire APP gene corresponding to NCBI database, accession no XM\_009710, comprising the Arctic mutation, i.e. nucleotide no. 2225 mutated from A to G, leading to an amino acid substitution from Glutamic acid to Glycine.
14. Antibodies against the A $\beta$  peptide according to claim 8.
15. A pharmaceutical composition, comprising the peptide according to claim 8 and physiologically acceptable excipients for human and veterinary use.
16. Use of the A $\beta$  peptide according to claim 8 for high throughput screening to find substances with anti-prototibrillar activity.
17. Method for prevention or treatment of AD, comprising the step: decreasing the formation of A $\beta$  prototibrils and/or lower meric forms thereof in a subject having, or suspected of having, AD.
18. A method according to claim 17, wherein said step is by active immunisation with a non wild-type prototibril or compound(s) with prototibril forming ability, wherein said prototibril or compound(s) have enhanced prototibril forming ability and/or enhanced immunogenicity compared to the wild-type counterparts.
19. A method according to claim 17, wherein said step is by passive immunisation with antibodies against a non wild-type prototibril or compound(s) with prototibril forming ability, such as A $\beta$ -Arc.
20. A method according to claim 17, wherein said step is by administration of agents with anti-prototibrillar activity.
21. A method according to claim 17, 18, 19 or 20, in combination with compound(s) having therapeutic benefits to AD patients

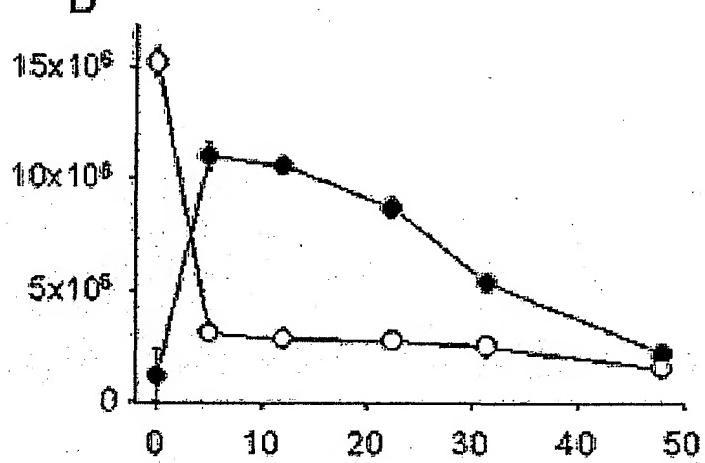
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Figure 1 A

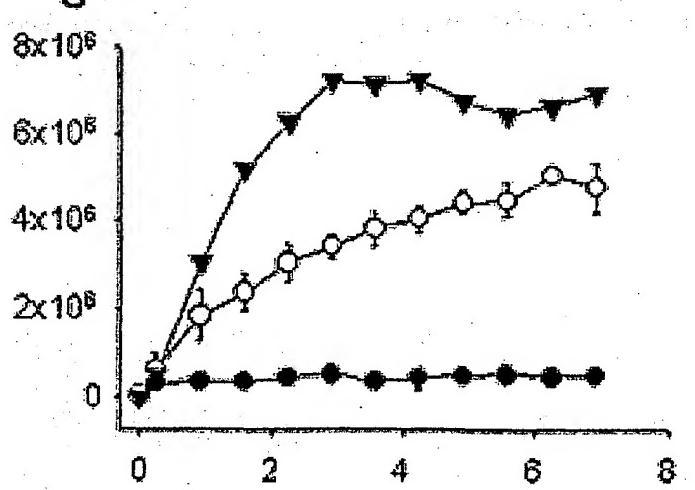


Absorbance units at 214 nm

B

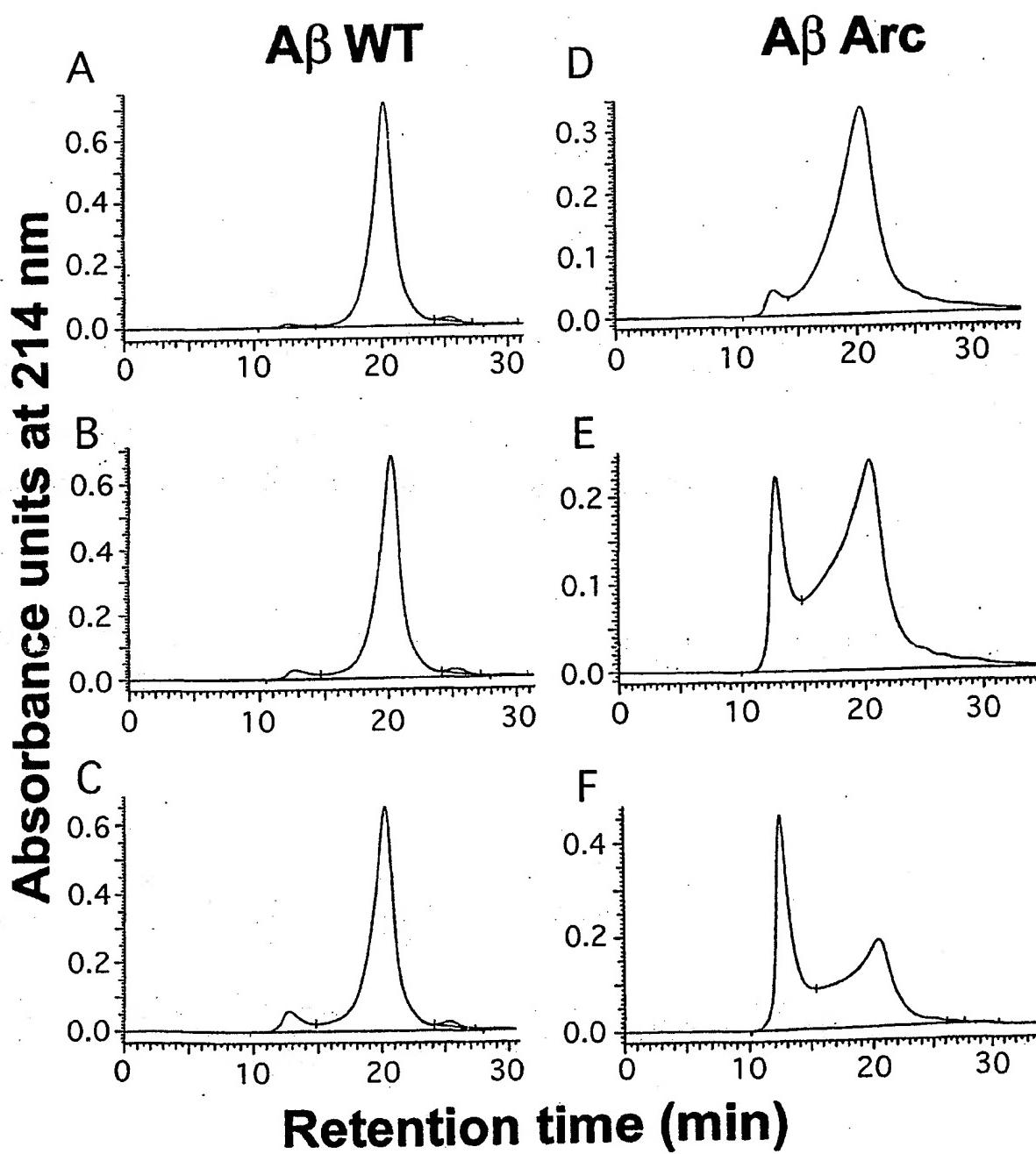


C



Time (hours)

- ▼▼▼ Protomembranous A $\beta$ 40Arc
- Protomembranous A $\beta$ 40wt+Arc (1:1)
- Protomembranous A $\beta$ wt

**Figure 2**

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(81) Designated States (national): AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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WO 02/03911 A3

(54) Title: PREVENTION AND TREATMENT OF ALZHEIMER'S DISEASE

(57) Abstract: The present invention relates to prevention and treatment of Alzheimer's disease (AD). More specifically, the invention relates to use of a non-wild type protofibril or compound(s) with protofibril forming activity for active immunisation in the purpose of treating or preventing AD. The invention further relates to a peptide,  $\alpha$ -Arc, with high protofibril forming activity as well as several applications thereof, such as antibodies against said peptide for passive immunisation against AD.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 01/01553

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC7: A61K 39/00, C07K 14/47, A61P 25/28**

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC7: A61K, C07K, C12N**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**SE,DK,FI,NO classes as above**

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**EPO-INTERNAL, WPI DATA, MEDLINE, BIOSIS, EMBASE**

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Nature, Volume 400, July 1999, Dale Schenk et al: "Immunization with amyloid-Beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse", pages 173-177  --	1-11,14-21
X	WO 0039310 A1 (THE UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC.), 6 July 2000 (06.07.00), page 12 - page 13; page 15 - page 16, example II and claims 38-44  --	1-11,14-21
X	US 5854204 A (FINDEIS ET AL), 29 December 1998 (29.12.98), column 63 - column 64, claim 2, SEQ ID No 1,3 and 14  --	1-5,7,14-21

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search

25 January 2002

Date of mailing of the international search report

04-02-2002

Name and mailing address of the ISA/  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 01/01553

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9927944 A1 (ATHENA NEUROSCIENCES, INC.), 10 June 1999 (10.06.99), page 14 - page 16, the claims  --	1-11,14-21
X	WO 9531996 A1 (MILKHAUS LABORATORY), 30 November 1995 (30.11.95), examples 1,7,8, claims 13-15  --	1-5,17
A	WO 9511994 A1 (ATHENA NEUROSCIENCES, INC.), 4 May 1995 (04.05.95), page 1, line 7 - line 15; page 5, line 31 - page 6, line 18; page 18, line 23 - page 19, line 30, the claims  --	1-11,14-21
P,X	WO 0072876 A2 (NEURALAB LIMITED), 7 December 2000 (07.12.00), page 16 - line 26; page 74 - line 81; page 98, the claims  -----	1-11,14-21

**INTERNATIONAL SEARCH REPORT**International application No.  
**PCT/SE01/01553****Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: **1-7, 17-21**  
because they relate to subject matter not required to be searched by this Authority, namely:  
**see next sheet Box I.1**
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

**see next sheet Box II**

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
**1, 3-5, 7 (partially), 2, 6, 8-11, 14-16 (immunization aspects searched)**

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.  
**PCT/SE01/01553**

Box I. 1

Claims 1-7, 17-21 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

.../...

**INTERNATIONAL SEARCH REPORT**International application No.  
**PCT/SE01/01553****Box II**

According to PCT Rules 13.1 and 13.2, an international application shall relate to one invention only or a group of inventions linked by one or more of the same corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art.

In Your application the following inventions have been found:

1. Use of a non-wild type protofibril or "compound(s) with protofibril forming ability" for immunization for prevention or treatment of Alzheimer's disease, according to claims 1,3-5 (partially) and 7 (partially).
2. Use, peptide, nucleic acid, vector and host cell comprising the peptide KLVFFAEDV or for immunization and prevention of Alzheimer's disease according to claims 2-11 (partially) and 14-16 (partially).
3. A transgenic non-human animal comprising a vector with the amyloid beta with the arctic mutation according to claims 12, 13 and 14-16 (partially).

The special technical feature of invention 1 is considered to be the general use of non-wild type protofibril or compounds with protofibril forming activity for immunization purposes in treatment of Alzheimer's disease. The special technical feature of invention 2 is considered to be the use of protofibril or compounds with the Arctic mutation Glu693Gly for immunization purposes in treatment of Alzheimer's disease.

Invention 1, 2 and 3 are not linked by a new common "special technical feature" as the use of beta amyloid 1-40 and/or 1-42 as an immunizing component has already been used as revealed by e.g. WO0039310 (as fusion protein with rubredoxin), Nature vol. 400, 1999, p173-7 and WO9927944 and transgenic animals with mutations of the human amyloid protein gene are known in the art.

Invention 1 has been searched and invention 2 to the extent the immunization aspects were involved.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

27/12/02

International application No.

PCT/SE 01/01553

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO	0039310	A1	06/07/00	AU 2486900 A	31/07/00
US	5854204	A	29/12/98	AU 5252496 A CA 2214247 A EP 0815134 A JP 11514333 T US 5817626 A US 5854215 A US 6303567 B US 6319498 B WO 9628471 A US 5985242 A	02/10/96 19/09/96 07/01/98 07/12/99 06/10/98 29/12/98 16/10/01 20/11/01 19/09/96 16/11/99
WO	9927944	A1	10/06/99	AU 4087999 A BG 104562 A BR 9815357 A CN 1281366 T DE 1033996 T EE 200000379 A EP 1033996 A EP 1079701 A HR 20000443 A HU 0100627 A NO 20002784 A PL 342649 A WO 9959427 A AU 1706199 A	06/12/99 31/01/01 24/10/00 24/01/01 07/06/01 16/04/01 13/09/00 07/03/01 31/10/00 28/06/01 31/07/00 18/06/01 25/11/99 16/06/99
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## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SE 01/01553

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 702293 B	18/02/99
		AU 8079894 A	22/05/95
		AU 8080994 A	22/05/95
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		DE 69426571 D,T	09/08/01
		DK 730643 T	14/05/01
		EP 0730643 A,B	11/09/96
		SE 0730643 T3	
		EP 0736106 A	09/10/96
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		US 5850003 A	15/12/98
		US 6245964 B	12/06/01
		WO 9511968 A	04/05/95
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WO 0072876 A2	07/12/00	AU 5316300 A	18/12/00
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60/217,098 10 July 2000 (10.07.2000) US

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(54) Title: PREVENTION AND TREATMENT OF ALZHEIMER'S DISEASE

(57) Abstract: The present invention relates to prevention and treatment of Alzheimer's disease (AD). More specifically, the invention relates to use of a non-wild type protofibril or compound(s) with protofibril forming activity for active immunisation in the purpose of treating or preventing AD. The invention further relates to a peptide, a<sup>β</sup>-Arc, with high protofibril forming activity as well as several applications thereof, such as antibodies against said peptide for passive immunisation against AD.

**AMENDED CLAIMS**

[received by the International Bureau on 5 March 2002 (05.03.02);  
original claim 1 amended; remaining claims unchanged (1 page)]

1. Use of a non-wild type protofibril or compound(s) with protofibril forming ability in the production of a drug for immunisation for prevention or treatment of Alzheimer's disease (AD).
2. Use according to claim 1, wherein said protofibril or compound(s) with protofibril forming ability comprises the following amino acid sequence KLVFFAEDV.
3. Use according to claim 1 or 2, wherein said protofibril or compound(s) with protofibril forming ability is mutated or modified in relation to corresponding wild-type counterparts.
4. Use according to claim 1, 2 or 3, wherein said protofibril or compound(s) with protofibril forming ability comprises an A $\beta$  peptide ( $\beta$ -amyloid protein).
5. Use according to claim 4, wherein said protofibril or compound(s) with protofibril forming ability comprises a A $\beta$  peptide related to AD.
6. Use according to claim 5, which is A $\beta$ -Arc as disclosed in SEQ ID NO 1.
7. Use according to any of the above claims, wherein said profibril or compound(s) with protofibril forming ability is used in combination with A $\beta$  peptides having mutations.
8. A peptide A $\beta$ -Arc having the amino acid sequence disclosed in SEQ ID NO 1 comprising a glycine at position 22 instead of glutamic acid compared to wild type A $\beta$  peptide.
9. Nucleic acid encoding the peptide according to claim 8.
10. Vector comprising the nucleic acid according to claim 9.
11. Host cell comprising the vector according to claim 10.
12. Transgenic non-human animal comprising the vector according to claim 10.

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